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Reference Ranges Of Haematological Parameter Concentrations Of Young Adult Males In Khartoum State.

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Abstract: Reference values of haematological parameters such as red blood cells count & haemoglobin (Hb)concentration of Sudanese population is not determined. The aim of this study is to determine the reference ranges of red blood cells count, haemoglobin concentration, haematocrit, MCV, MCH, MCHC and RDW among Sudanese young adult males and compare them to international and regional reference intervals used in practice. This gives an insight towards further setting reference values to Sudanese population in the future. This is a cross sectional study conducted in Khartoum state, Sudan. About 200 male students from three higher educational institutes were selected by a convenient sampling method. The age of study participant was 18 - 25 year. Venous blood samples were collected in EDTAK3 containers for the blood test. RBC's count, Haemoglobin concentration, Haematocrit, Hb, MCV, RDW were measured for each participant. IBM.SPSS version 21 statistical software was used for data analysis, P< 0.05 was assumed as significant and 95% confidence level was accepted. The results were displayed as mean, standard deviation (SD) and range for each haematological parameter. RBCs 5.29±.41 cell1012/L (4.45 – 6.13), haemoglobin concentration 15.1±1.06 g/dl (12.5 – 17.49), haematocrit 45.94±3.1 % (40.1 – 53.19), MCV 86.92±4.33 fl (77.81 – 95.79), MCH 28.58±1.92 pg (23.72 – 32.29), MCHC 32.9±1.29 g/dl (29.41 – 34.89), RDW-SD 44.86±3.57 fl (38.8 – 52.69) and RDW-CV 13.68±1 % (12 – 15.69). All parameters compared to American ones revealed statistically significant differences. All measured parameters were significantly different from parameters of other African populations. Worldwide used reference intervals are inapplicable in Sudan as haematological parameters showed statistically significant variations.

Key words: Reference range, RBC's count, Haemoglobin concentration, Haematocrit, Hb, MCV, RDW.

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1. INTRODUCTION

Reference value is the value that is obtained by frequent observation provided that the subject under the study is normal.[1]Reference values of haematological parameters are of great importance because they are used by clinicians for diagnostic and therapeutic purposes.[2]The reference values for haematological parameters vary due to many factors including ethnicity, age, sex, Body Mass Index (BMI), genetics, and nutrition, along with social and environmental factors. [3-5]The currently used reference ranges of haematological parameters in clinical practice in Sudan and many other African countries are the American normal reference ranges or other white population derived values or may even Sysmex derived values. This practice has been questioned by many studies which reveal significant differences in the normal ranges of haemoglobin, red blood cell count and red blood cell indices of Africans and those of whites. According to one study, 19.8% of African American women and 17.7% of African American men would have been categorized as anaemic patients compared with 5.3% of white women and 7.6%. of white men respectively when applying the American reference ranges.[6] A lot of studies have been done in African countries to establish reference values for their population and showed significant differences.[7-11]In Botswana, the results obtained significantly differ from commonly used intervals in practice especially for haemoglobin concentration.[8]A survey in Tanzania which measured haematological parameters and compared it with Sub Saharan African countries and other countries reference values used in practice concluded that most of the parameters are inapplicable due to huge variations.[9,10] In Uganda they found that haemoglobin concentration, RBCs count, haematocrit level and (Mean Cell Volume) MCV are lower than standard reference values.[11]In Ethiopia, the haematological variables obtained were significantly different from the reference range used in clinical practice.[12] Studies in Sudan showed significant differences also. Hamad &Musa (2006) reported that haemoglobin concentration in Sudanese children is considerably lower than the WHO standard one and American reference range. Haemoglobin concentration even differs between Sudanese tribes.[13] In about 50% of the Sudanese children within the age group of 12-14 years have haemoglobin values less than 12g/dl, while 6.3% showed a value of 9.4g/dl or less without any symptoms of anaemia. WHO stated that 12 gm/dl is the cutoff point to diagnose anaemia for this age group.[14] Southern tribes have the lowest haemoglobin value, while Kordofan tribes have the highest haemoglobin concentration.[13] Outside Africa, similar findings have been obtained. A study from Malaysia, detected considerable differences in the reference values of haematological parameters of Malaysian population from manufacturers, western population or laboratory manuals.[15] A study in Saudi Arabia revealed lower levels of haematological parameters than reported parameters in international studies. The study found that even region affects the haematological values.[16]

It is clear that the current use of reference ranges of RBCs count, RBCs indices and haemoglobin which are derived from different populations (American and European), or from reference intervals set by the device used need to be verified by proper studies which should aim to set the specific reference values of the local population in question. This study aims to establish reference ranges for RBC's count & indices, haemoglobin (Hb), haematocrit and Red cell Distribution Width (RDW) in young adult males in Khartoum state, Sudan.

2. MATERIALS AND METHODS

2.1 Study Participants/Study Period

The study was a cross sectional study conducted in Khartoum state within the period of seven months from January 2020 to July 2020. Sample size was determined using an appropriate formula for single population mean to get a representative number of participants. The formula was used as:

$$4^* (z\alpha/2)^{2*}(\delta)/ d^2$$

d= margin of error one is willing to tolerate on the result, = 0.5 at 95% confidence interval.

Z α/2 = confidence level obtained from z1 table, = 1.96 value at 95% confidence interval (CI)

δ = measure of variation from the mean = 3.29. The above formula gave a sample size of 200.

The study participants were recruited from three higher education institutions at Khartoum state; University of Khartoum; AL-Neelain University and Al-Nahda College. About 200 apparently healthy adult males were included in the study. Medical history and examination were used to select apparently healthy individuals and to identify those with chronic illnesses, haematological diseases, or who had recent illnesses and those with family history of haematological diseases as they were excluded from the study. Smokers, alcoholics, those with a history of blood donation within the previous 3 months were also excluded. Informed consents were signed by participants following detailed explanations of the aims of the study. A questionnaire was filled out for each participant and included sociodemographic information and anthropometric measurements. About1.5-2 ml blood samples were taken from each participant through a vein puncture using a needle and syringe then transferred into EDTAK3 containers and sent for laboratory investigations in AL-TIGANA lab in Khartoum. Device used for analysis is SYSMEX KX 21 haematological automated analyzer. Participant's confidentiality was secured throughout the process of data collection, laboratory investigations and analysis.

3. DATA ANALYSIS

Data were analyzed using SPSS program version 20. The mean± standard deviation and median were obtained for all study variables. Reference ranges for hematological parameters were measured as (2.5^{th}—97.7^{th}) percentiles at confidence level 95%. Two tests of significance were used: the independent one sample t-test for comparing means, and Wilcoxon-Rank Signed test to compare medians between this study variable and other studies ones (at confidence level 95%), P value<0.05 is considered statistically significant. One-Sample Kolmogorov-Smirnov test of normality was used to test normal distribution.

4. ETHICAL CONSIDERATION

Ethical approval was obtained from the ethical committee of faculty of medicine, University of Khartoum with the reference number FM/DO/EC.

5. RESULTS

The study included 200 adult males. Their age ranged between 18 – 25 years, with a mean age of 20.4 ±2.2 years.

Age and other anthropometric characteristics and blood pressure of the study population were summarized in table 1.

Table 1: Anthropometric measurements and blood pressure.

N=200	Mean (SD)	Median	Minimum	Maximum
Age (years)	20.4 (2.2)	20	18	25
Weight (kg)	69.1(13.2)	68	44.1	122.5
Height (cm)	175.5 (6.9)	176	153	195
BMI (kg/m^2)	22.4 (3.9)	21.2	14.9	40.93
Systolic blood pressure	118.5 (7.1)	120	100	140
Diastolic blood pressure	76.7 (5.9)	80	60	100

Test of normality was performed for all the study variables using the Kolmogorov-Smirnov test and all data were normally distributed except Age, systolic blood pressure, diastolic blood pressure, BMI and MCHC. Descriptive statistics of the main study parameters are shown in table 2. The mean RBCs count was 5.29±.41cells 10^{12}/l, the mean haemoglobin concentration was 15.1±1.06 g/dl, and the mean haematocrit was 45.94±3.11 %. Reference ranges of RBCs, haemoglobin, haematocrit, MCV, MCH, MCHC RDW-SD have been determined by 2.5th—97.5th percentiles (Table 2).

Table 2: Descriptive statistics of the study's haematological parameters. Ranges are expressed as 2.5 – 97.

Hematological parameter	Mean (SD)	SE	Median	Minimum	Maximum	Reference range
RBCs (cell10^{12}/L)	5.29 (0.41)	0.02	5.29	4.35	6.69	4.45 – 6.13
Haemoglobin (g/dl)	15.1 (1.06)	0.07	15.15	11.3	18.3	12.5 – 17.49
Haematocrit (%)	45.94 (3.11)	0.22	45.8	36.8	57	40.1 – 53.19
MCV (fl)	86.92 (4.33)	0.30	86.8	71.3	102	77.81 – 95.79
MCH (pg)	28.58 (1.92)	0.13	28.8	20.4	34.1	23.72 – 32.29
MCHC (%)	32.9 (1.29)	0.09	33	22.7	36.7	29.41 – 34.89
RDW-SD (fl)	44.86 (3.57)	0.25	44.3	37.7	54.9	38.8 – 52.69

Mean- RBCs, haemoglobin, haematocrit, MCV, MCH, and RDW were compared to corresponding American values using independent one sample t-test. (Mean Corpuscular Haemoglobin Concentration)MCHC, however, is compared using Wilcoxon signed-rank test by median because of its abnormal distribution. All data showed significance differences (P value=0.001) (Table 3).

Table 3: Comparison of the reference ranges for haematological parameters from this study with the American reference ranges.

Parameter	Study reference range	Mean	Median	American reference range (17)	Mean*	Median*	**P value
RBCs (cell10^{12}/L)	4.45 – 6.13	5.29	5.29	44.4 – 5.84	5.11	5.08	0.001
Haemoglobin (g/dl)	12.5 – 17.49	15.1	15.15	13.7 – 17.2	15.41	15.46	0.001
Haematocrit (%)	40.1 – 53.19	45.94	45.8	41 – 50	45	45	0.001
MCV (fl)	77.81 – 95.79	86.92	86.8	81.1 – 96.6	88.9	88.9	0.001
MCH (pg)	23.72 – 32.29	28.58	28.8	27.2 - 33	30.2	30.2	0.001
*MCHC (%)	29.41 – 34.89	33	*33	32.3 – 35.5	*34	33.9	0.001
RDW-CV (%)	12 – 15.69	13.68	13.6	11.6 - 14	12.6	12.6	0.001

Ranges are expressed as 2.5 – 97.5 percentiles. * American corresponding paramet ** Comparison of median using non-parametric t-test The same comparisons, except for the RDW, were performed using Ethiopian values as shown in table 4 and Botswana values as shown in table 5 . All variables showed significant differences.

Table 4: Comparison of the reference ranges for haematological parameters from this study with the reference ranges from Ethiopia. Ranges are expressed as 2.5 – 97.5 percentiles.

Variable	Study reference ranges	Mean	Median	Ethiopian reference ranges (12)	Mean*	Median*	**P value
RBCs (cell10^{12}/L)	4.45 – 6.13	5.29	5.29	4.05 – 6.46	5.50	5.58	0.000
Haemoglobin (g/dl)	12.5 – 17.49	15.1	15.15	12.21 – 17.7	15.38	15.6	0.000
Haematocrit (%)	40.1 – 53.19	45.94	45.8	37.47 – 56.6	48.45	48.9	0.000
MCV (fl)	77.81 – 95.79	86.92	86.8	81.49 – 96.4	88.8	88.9	0.000
MCH (pg)	23.72 – 32.29	28.58	28.8	25.4 – 30.95	28.26	28.3	0.017
*MCHC (%)	29.41 – 34.89	32.9	33	29.05 – 34.14	31.83	32	0.000*

* American corresponding parameter ** Comparison of median using non-parametric t-test.

Table 5: Comparison of the reference ranges for haematological parameters from this study with the reference ranges from Botswana. Ranges are expressed as 2.5 – 97.5 percentiles.

Variable	Study reference ranges	Mean	Median	Botswana reference ranges (8)	Mean	median	*P value
RBCs (cell10^{12}/L)	4.45 – 6.13	5.29	5.29	4.2 – 6.3	5.2	5.18	0.001
Haemoglobin (g/dl)	12.5 – 17.49	15.1	15.15	11.9 – 17.1	15.12	15.3	0.796
Haematocrit (%)	40.1 – 53.19	45.94	45.8	36.1 – 49.3	43.38	43.3	0.001
MCV (fl)	77.81 – 95.79	86.92	86.8	73.8 – 95.6	83.86	84	0.001
MCH (pg)	23.72 – 32.29	28.58	28.8	23.4 – 33.1	29.25	29.5	0.017
*MCHC (%)	29.41 – 34.89	32.9	33	31.8 – 37.6	34.9	35	0.001*

* Comparison of median using non-parametric t-test.

6. DISCUSSION

Accurate clinical diagnosis and management often require laboratory investigations. Laboratory test interpretation on the other hand is dependent on the availability of accurate reference ranges. Reference ranges of haematology parameters are of great importance due to their frequent use in disease diagnosis and management.[18, 19] Reference ranges are varied by age, ethnicity, gender, and geographical location. [3, 4,5] Smoking and even periodontal health are also reported to affect haematological parameters.[17] It is very important that each population establish its own reference.[20] This study determined reference ranges for the following haematological parameters: RBCs count, haemoglobin concentration, hematocrit, MCV, MCH, MCHC and RDW-SD. The reference intervals were determined using the 2.5th and 97.5th percentiles. The present study detected major differences with similar studies conducted in various parts of the world, including Africa, and America. RBCs count is significantly higher in the study participants than means from American and Botswana (P <0.001) but lower count than Ethiopian (P =0.001).[8,12, 17] Mean haemoglobin concentration is significantly lower than American and Ethiopian values (P <0.001), but it was similar to values obtained from Botswana (P = 0.995). [8,12, 18] Haematocrit is significantly higher than means from America and Botswana (P <0.001) but significantly lower than Ethiopian (P =0.001). [8,12, 18] It was noted that Ethiopian values of RBCs, haemoglobin concentration and haematocrit were higher compared to other African studies. This is probably due to the effects of living at high altitudes. RBCs indices: MCV, MCH, MCHC also showed significant differences with indices derived from other populations. MCV was significantly lower than American and Ethiopian values but significantly higher than Botswana (P = 0.001). [8,12, 18] On the other hand, MCH and MCHC were significantly lower than American values and values from Botswana but significantly higher than Ethiopian values (P < 0.05). [8,12, 18] This should consider redefining microcytic and hypochromic terms in Sudan and in Africa as a whole as American western values appear inapplicable. RDW-SD mean was found to be 44.86fl (38.8 – 52.69) while RDW-CV mean was 13.68% (12 – 15.69). Along with RBCs indices, they are very important in diagnosing causes of anaemia. RDW is preferred on indices for detection of early changes. African studies did not have results for RDW but when compared to American mean, RDW-CV was significantly higher (P = 0.001).[18] The study confirms many other studies which detect significant differences in haematological reference values of western population from African population. Beutler E, West C (2005) reported that the average results of haemoglobin, mean corpuscular volume (MCV), and white blood cell count of African-Americans differ from those of whites significantly.[6] A significant proportion of African American women would have been diagnosed as anaemic patients when reference ranges of whites were applied. [6] Another study confirms that African American children and adolescents have lower mean haemoglobin values than do whites.[19] A recent study in the Northwest region of Cameroon, found that the currently used haematological reference intervals do not represent the population of the region and call for establishment of reference intervals applicable to each Cameroonian region.[20] Addai-Mensah O et al (2019) compared haematological values of three regions in Ghana with values of Caucasian, other African and Western countries and they reported significant intraregional and interregional as well as international variations of haematological reference ranges in the populations studied. [21] Moreover, they concluded that each geographical area should establish geography-specific reference ranges if accurate diagnosis and proper clinical decisions are to be made. [21] Similar results were reported elsewhere throughout Africa[22, 23]

Outside Africa, many studies support our findings and reported similar differences in haematological parameters.[15, 16, 24] Studies from Malaysia, Saudi Arabia and Oman all detected significant variation from other international reference intervals which are usually lower than the used reference ranges in clinical practice.

7. CONCLUSION

Sudanese population has haematological parameters that significantly vary from American and regional African reference values and hence they are not applicable to our population. Many studies have detected variation in hematological values between nations and even between tribes and regions of a single nation.

Age and gender based as well as tribe specific and region-specific hematological reference intervals should be established for Sudanese population provided that the current study detected significant differences from what is being applied now in clinical practice. Establishment of these haematological reference values will surely help clinicians to reach accurate diagnosis, treatment and follow up and limit unnecessary workup. The current study did not include the female population of Khartoum, so it does not assess sex-specific differences. The study also did not include pediatric and geriatric populations. Despite these limitations, the advantages of this study remain unmasked.

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9. CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

10. AUTHORS CONTRIBUTION STATEMENT

Dr Mustafa and Dr Abdalla conceptualized and collected the data. Dr. Nisreen Daffa Alla and Dr Rehab Badi analyzed these data and designed the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

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